

The Effect of Phosphogypsum on the Activity of *Desulfotomaculum ruminis* in Lactate Medium

A. Juszczak, M. Waligórska, K. Seifert, A. Meller, M. Habrych, F. Domka

Faculty of Chemistry, Adam Mickiewicz University,
Grunwaldzka 6, 60-780 Poznań, Poland

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Abstract

The influence of phosphogypsum (a waste product of phosphoric acid production), on the activity of *Desulfotomaculum ruminis* bacteria in a lactate medium was tested. In order to establish the optimum conditions of the reduction of sulphates present in the phosphogypsum, the influence of the concentrations of phosphogypsum, organic substrate and inoculum in the medium on the degree of conversion was studied. It has been shown that phosphogypsum present in the medium at a concentration of up to 20 g/dm³ is not toxic towards the bacteria and can be used as a source of assimilative sulphates. In the optimum conditions of the process (the concentrations of phosphogypsum 10g/dm³, lactate 20.32 g/dm³, inoculum 4%v/v) the conversion of sulphates to sulphides after 7 days reaches over 92%.

Keywords; phosphogypsum, sulphate reducing bacteria (SRB), *Desulfotomaculum ruminis*, dissimilatory sulphate reduction

Introduction

Utilisation of phosphogypsum has for a long time been an important problem for the producers of phosphoric acid all over the world and at the same time a great challenge to ecologists and all those concerned with disposal of waste products. Its hazardous effect on the natural environment is related to its acidic character and radioactivity [1, 2].

Phosphogypsum is the waste product in production of phosphoric acid from phosphorites and apatites by extraction with sulphuric acid. The main component of this waste product is gypsum or bassanite (-95%), whose degree of hydration and grain size depend on the conditions of the process of production. Phosphogypsum also contains celestite and (up to 3%) insoluble components of ore, trace amounts of sulphuric acid, and non-eluted phosphoric acid. The phosphogypsum obtained from the processing of phosphorites contains much more radioactive elements and rare earth elements (lanthanide, uranium, radium and thorium) than that obtained from apa-

tite processing. The actual content of these elements depends on the particular method of processing [3, 4].

The worldwide demand of phosphoric acid, used for production of fertilisers, plastifiers and detergents, has led to the accumulation of great deposits of phosphogypsum. It is estimated that only 3% of world phosphogypsum wastes are utilised, and production of 1 ton of P₂O₅ gives about 4 tons waste [5]. At the chemical plant in Police, there is at present about 20 million tons of phosphogypsum and this amount will increase each year by about 2 million tons.

Hitherto a number of methods of phosphogypsum utilisation have been proposed, of which the most promising involve conversion of this waste into cement and sulphuric acid [6] or chalk and ammonium sulphate, or its use as a component of building materials [7]. Unfortunately, these solutions are often not economical. A promising solution seems to be its microbiological utilisation based on the use of sulphate reducing bacteria (SRB) [1, 5, 8]. As a result of this SRB activity phosphogypsum undergoes biotransformation into sulphate and calcium ions. The obtained calcium ions could be the raw product for production of chalk or calcium while the sulphate

ions could be oxidised with the use of aerobic bacteria *Thiobacillus ferrooxidans*, *Thiobacillus thiooxidans* into sulphuric acid or elementary sulphur [5, 9].

One of the most common and best known bacteria involved in the process of desulfurication are the bacteria from the genus *Desulfovibrio* and *Desulfotomaculum* [10, 11]. These mesophylic organisms (the optimum temperature of growth 28-38°C), living in neutral pH (the optimum pH 7.0-7.8) can use in their metabolism many different organic compounds and occur in almost all kinds of environments, so have found application in many branches of the economy [12-16].

This paper reports results of a study aimed at establishing the optimum conditions of biotransformation of phosphogypsum conducted with the use of *Desulfotomaculum ruminis*. This study is a part of our comprehensive investigation undertaken to find a method of bi-oultimisation of this waste product, carried out within a larger project on processing wastes from the agricultural and food industries [17].

Materials and Methods

Micro-organisms

Desulfotomaculum ruminis bacteria were grown in a modified Starkey medium [18] at 37°C and pH 7.0, containing 4% v/v inoculum. Particular portions of phosphogypsum were flooded with the medium devoid of all sulphates.

Table 1. Physical and chemical characterisation of phosphogypsum from Police Chemical Plant.

Component	Content [% weight]
SO ₄ ²⁻	59.0
PO ₄ ³⁻	1.60
Ca ²⁺	20.0
Mg ²⁺	0.06
Fe ³⁺	0.04
H ₂ O	17.0
Contents solubility at 25°C = 2.81 g/dm ³	

Phosphogypsum

Samples of phosphogypsum were collected from the waste dumping grounds at the Police Chemical Plant. These were the wastes after production of phosphoric acid from apatites and phosphorites imported from Morocco and Tunisia. Before introduction into the reactors, the samples were ground and sieved through 0.315 mm mesh. Chemical composition of phosphogypsum was established using the following methods: sulphates were determined by the weighing method [19], calcium by the complexometric method [20], phosphates by the spectrophotometric method [21], and iron and magnesium by the ASA method (on a spectrophotometer ELA Unicam SP 90A).

The content of water was determined after drying of the phosphogypsum samples for 1 hour at 110°C. The weighted portions of phosphogypsum used in the study

Table 2. Characteristics of the process of bioreduction of sulphates contained in the phosphogypsum from Police Chemical Plant (4% v/v inoculum, *Desulfotomaculum ruminis*, 37°C).

Concentration of phosphogypsum in the medium [g/dm ³]	Initial concentration of sulphates [gS-SO ₄ ²⁻ /dm ³]	Concentration of lactate [gC/dm ³]	C/S ²⁻ - ratio	Proces duration [h]	Concentration of sulphides after the process [gS/dm ³]	Degree of reduction SO ₄ ²⁻ → S ²⁻ [%]	Decrease of ChZT [gO ₂ /dm ³]
5	0.98	3.2	3.4	144	0.92	93.9	3.2
		4.8	5.1*	120	0.95	96.9	3.6
		6.4	6.9*	120	0.93	94.9	3.4
10	1.96	3.2	3.4	144	0.93	47.4	3.1
		4.8	3.3	168	1.45	75.0	5.7
		6.4	3.5	192	1.81	92.3	5.8
15	2.94	3.2	3.4	132	0.93	31.6	3.2
		4.8	3.3	156	1.46	49.7	5.8
		6.4	3.3	180	1.95	66.3	6.3
20	3.92	3.2	3.4	132	0.93	23.7	3.0
		4.8	3.2	156	1.51	38.4	5.8
		6.4	3.3	200	1.95	50.0	6.0

* excess of organic carbon

were: 5,10,15 and 20 g/dm³. The portions were placed in the medium free from sulphates in individual reactors, after pH was established (usually 10 minutes) the value of pH was adjusted to 7.0 by IN NaOH.

Kinetic Study

Kinetic measurements were conducted in sealed reactors of 250 ml capacity containing particular portions of phosphogypsum and the medium free from sulphates

with 4% inoculum. The pH of the mixture was adjusted to 7.0. At certain periods of time the reactors were blown with helium and the produced hydrogen sulphide was absorbed in absorption bulbs containing 0.2 M solution of cadmium acetate. The amount of sulphates formed was determined as a sum of the sulphates precipitated in the reactor and the cadmium sulphide precipitated in the absorption bulbs.

In 24 hours the precipitate was deposited in the reactors in the amount of 0.048 g S²⁻/dm³, irrespective of the amount of phosphogypsum, and was identified as Fe₂S₃.

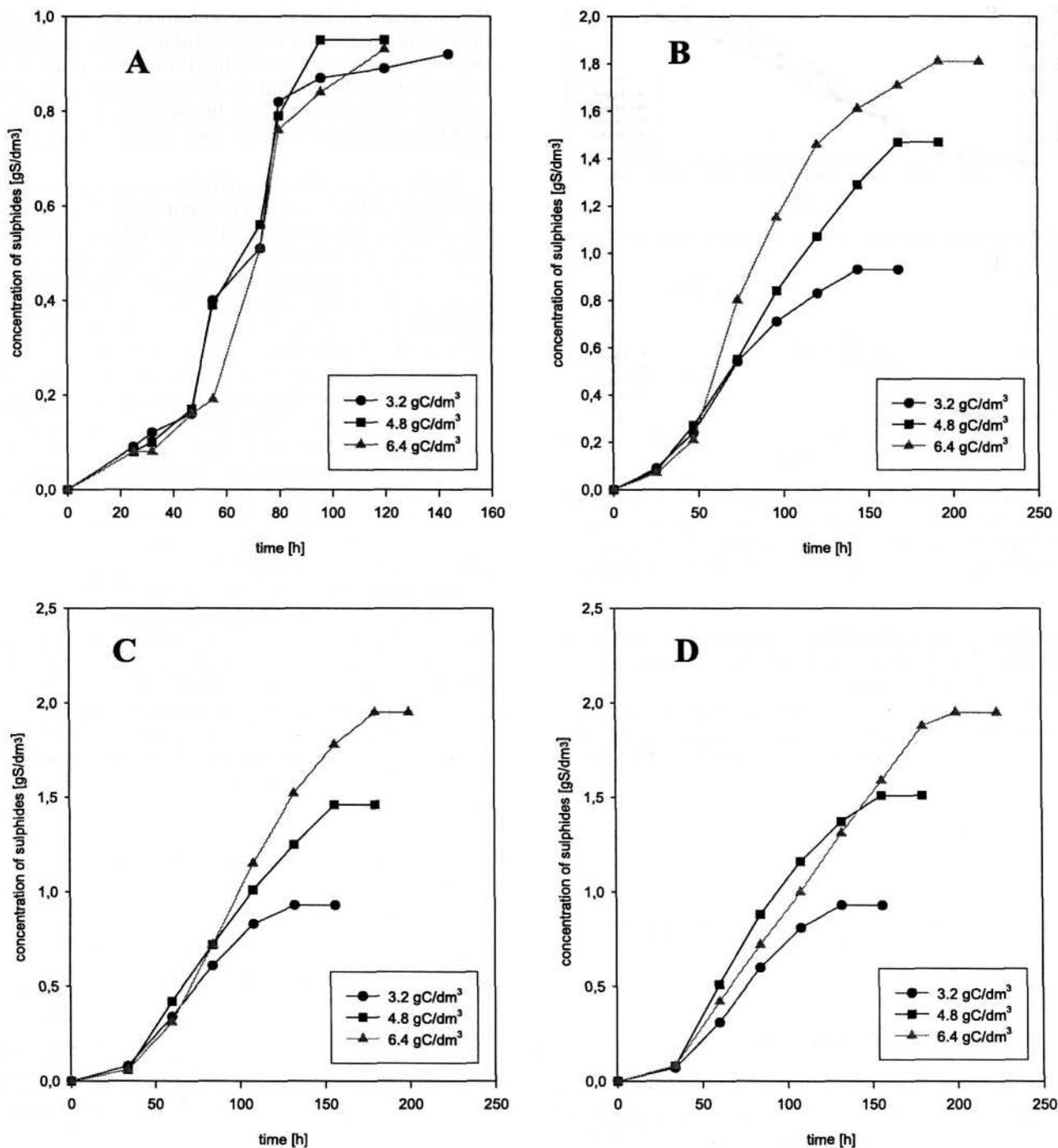


Fig. 1. The influence of the initial concentration of lactate in the medium on the reduction of sulphates contained in the phosphogypsum for different contents of phosphogypsum in the medium: A - 5 g/dm³; B - 10 g/dm³; C - 15 g/dm³; D - 20 g/dm³.

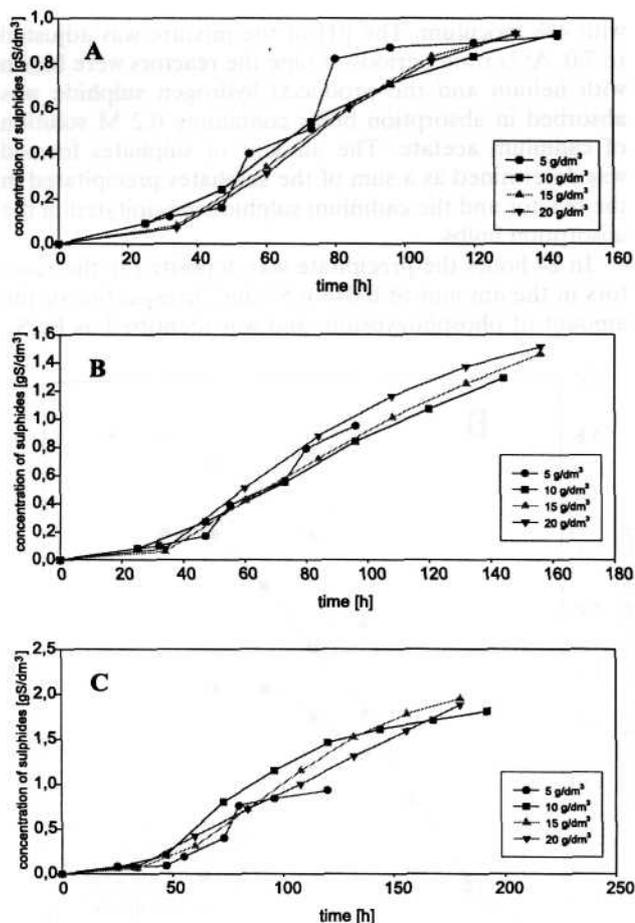


Fig. 2. The influence of the initial concentration of phosphogypsum in the lactate medium on reduction of sulphates contained in the phosphogypsum for different contents of sodium lactate in the medium: A - 3.2 g C/dm³; B - 4.8 g C/dm³; C - 6.4 g C/dm³.

Its amount was limited by the content of Fe³⁺ ions in the medium. The sulphides were determined using the iodometric method by titration.

In the series of measurements testing the influence of organic substrate on the course of the process we used lactate in the concentrations of [g/dm³] 10.16, 15.24, 20.32.

ChZT

The index describing the contents of organic matter before and after the process was determined by titration using dichromate [20]. The results are mean values of at least three measurements.

Results and Discussion

The chemical composition of the phosphogypsum samples studied is displayed in Table 1. The solubility of the phosphogypsum at 25°C was 2.81 g/dm³, converted to dry mass.

Kinetic measurements were conducted in a few variants: for four different contents of phosphogypsum in the

media and for three concentrations of lactate, see Figs. 1 and 2. The kinetic curves describing biotransformation of phosphogypsum have similar shapes corresponding to three typical phases of micro-organism growth lag, phase of the logarithmic growth and the stationary phase. In all cases the induction period took about two days (48 h); an increase of the inoculum from 4% to 10% v/v had no effect on the process.

In the medium containing 5g of phosphogypsum per dm³ with lactate at a concentration of 10.16 g/dm³ (3.2 gC/dm³) practically the whole content of sulphates (95% on average) was reduced within 5 days (Fig. 1a). A further increase of lactate concentration did not increase the rate of the process (Fig. 1a, Table 2). This means that the molar ratio of the introduced organic carbon to the reduced sulphide sulphur of 3.4 proved sufficient to ensure the optimum course of the process. In this variant ChZT decreased by 3.2 g CVdm³, which corresponds to 42%.

In the sample with phosphogypsum in the concentration of 19 g/dm³ the process significantly depended on the concentration of lactate (Fig. 1b, Table 2). The higher the concentrations of lactate, the higher the ratio of the process and the greater the total amount of sulphates reduced. The results were from 0.93 g of sulphide sulphur/dm³ for 10.16 lactate (3.2 g C/dm³) to 1.81 g sulphide sulphate /dm³ for 20.32 g/dm³ lactate (6.4 g C/dm³), for which the degree of reduction was over 92%. The molar ratio of carbon introduced to the reduced sulphide sulphur was similar - from 3.3 to 3.5. In this series of experiments ChZT was reduced by 42% on average. The duration of the process increased with increasing amount of the sulphates reduced, from 6 days for 0.93 g of the reduced sulphide sulphur/dm³ to 8 days for 1.81 g of the reduced sulphide sulphur/dm³. A further increase of the concentration of phosphogypsum to 15 g/dm³ and then 20g/dm³ applied with the same three values of lactate concentrations resulted in an insignificant increase of the sulphide sulphur reduced for the highest concentration of lactate. For instance, to reach 1.95 g of sulphide sulphur/dm³, this increase was only 8% (Figs 1c, 1d, Table 2). The molar ratio of the introduced carbon to the reduced sulphur was 3.3 - 3.4, and the mean ChZT decrease was 45%. The duration of the process was similar to the sample with phosphogypsum at a concentration of 10 g/dm³.

Table 3. The course of bioreduction of sulphates contained in phosphogypsum after repeated addition of organic carbon (lactate) and inoculum (4% v/v) (*Desulfotomaculum ruminis*, 37°C).

Process duration [h]	Content of phosphogypsum in the medium [g/dm ³]	Concentration of lactate in the medium [gC/dm ³]	Content of S-S ²⁻ [g/dm ³]
68	15.0	4.80	0.31
96	20.0	6.40	0.79

Fig. 2 also illustrates the effect of the initial concentration of phosphogypsum in the media containing lactate at the following concentrations: 10.16 g/dm³

(3.2 g C/dm³), 15.24 g/dm³ (4.8 g C/dm³), and 20.32 g/dm³ (6.4 g C/dm³). On the basis of the curves the concentration of sulphides (g S/dm³) at a certain duration of the process can be determined.

The effect of impurities present in the medium on the degree of bioreduction of phosphogypsum was also tested. As follows from the results, a twofold increase of the concentrations of iron, magnesium and phosphorus in the medium did not change the kinetics of the process.

Attempts were made to repeat the process with the same samples, that is after the completion of the process the samples were inoculated (after pH adjustment) once again. All these attempts failed.

In another series of experiments, to the samples containing initially 15 and 20g of phosphogypsum/dm³ and 2.32 g/dm³ of lactate (6.4 g C/dm³) where the process has already ceased we added lactate in the amounts of 15.24 g/dm³ (4.8 g C/dm³) and 20.32 g/dm³ (6.4 g C/dm³); respectively, inoculum and pH was adjusted. In these conditions in the medium containing 15g of phosphogypsum and 15.22 g/dm³ lactate the degree of sulphates reduction to sulphides increased by over 10% (to -80%) after three more days of the process. In the sample containing 20 g phosphogypsum/dm³ and 20.32 g lactate/dm³, the degree of reduction increased by about 20% (to -70%) after an additional four days of the process (Table 3).

The insoluble products of the process making about 46% of the precipitate relative to the initial weighted portion of phosphogypsum were found to contain the following: 0.7% Mg²⁺, 1.25% Fe³⁺, 23.4% Ca²⁺, 8.5% PO₄³⁻, 0.75% S²⁻, and 9.1% of the mineral residue insoluble in acids. The remaining part of the products -90% is made of organic substrate, including biomass [22].

As follows from our results, on average in one day the process maintained in the phase of the logarithmic growth of the bacteria can give about 0.30 g of sulphides in 1 dm³ of the medium containing from 5 to 20g of phosphogypsum.

The experiments have shown that the phosphogypsum from the Police Chemical Plant is not toxic towards the sulphate-reducing bacteria used and, quite contrary, can be used as a source of sulphates assimilative by the bacteria. In all samples studied the molar ratio of the introduced organic carbon, originating from lactate, to the sulphides formed as a result of the process has been almost the same (3.2 - 3.5), and about 40% of the introduced organic carbon has been found to undergo total oxidation (a decrease in ChZT). The results have shown that under optimum conditions, in 9 days the process gives about 2 g of sulphide sulphur in 1 dm³, which corresponds to about 6 g SO₄²⁻/dm³ and about 10.5 g/dm³ of phosphogypsum.

The study of the process is continued and the efforts are directed towards finding the most economic source of carbon among the wastes of agricultural and food industry. Tests are also being continued with the use of other sulphate-reducing bacteria and mixed micro-organisms.

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